UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
[illegible], 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM **DATE: November 21, 2011**

SUBJECT: **Review of Submission Related Data packages DP387914 for Mancozeb, Chemical Number 014504**

FROM: **Valerie Woodard, Biologist**
Environmental Risk Branch V
Environmental Fate and Effects Division (7507P)

THRU: **Mah Shamim, Branch Chief**
Environmental Risk Brach V
Environmental Fate and Effects Division (7507P)

TO: **Christina Scheltema, Risk Manager Reviewer**
Mike Goodis, Risk Manager (RM 53)
Pesticide Re-Evaluation Division (7508P)

We have reviewed the following information submitted for Mancozeb (PC Code 014504). Completed DERs for these studies are attached.

GUIDELINE	BEAN	MRID	CLASSIFICATION
850 2200 Avian Acute Dietary Toxicity-Bobwhite Quail	D387914	484178-01	ACCEPTABLE
850 2200 Avian Acute Dietary Toxicity-Mallard Duck	D387914	484178-02	ACCEPTABLE

D387914: MRID 484178-01 Bobwhite Quail *(Colinus virginianus)*

ENDPOINT	LC50	NOAEC
8 Day Survival	Not estimated. No mortality for any tested concentrations.	
Change in Body Weight		1000 ppm for body weight

The information provided resulted in an ACCEPTABLE classification using Bobwhite quail.

A dietary toxicity study using Bobwhite quail was conducted to determine the effect of Ethylenethiourea (ETU) on birds. A total of 10 animals per treatment were administered a diet of ETU for five days (D0-D5). The birds were observed for three days following exposure (Day 6-8). Measured concentrations (in parenthesis) as a percent of nominal were 99 (537 ppm a.i.), 97 (973 ppm a.i./L), 97 (1,780 ppm a.i./L), 97 (3,070 ppm a.i./L) and 98% (5,540 ppm a.i./L).

During acclimation, all birds were observed daily. Test birds were observed four times on the day of test initiation and twice daily through the rest of the test. Individual body weights were measured at test initiation (D 0), Day 5 and test termination (D 8).

No LC_{50} was calculated because no mortality was reported for any treatment group. Body weight data were compared using Bonferroni t-test in TOXSTAT® software.

D387914: MRID 4841787-02 (Mallard Duck)

ENDPOINT	LC50	NOAEC
Survival (5 day exposure plus 3 day post-exposure duration)	Not estimated. No mortality for any tested concentrations.	
Change in Body Weight		NOEC is less than the lowest tested concentration. All treatment concentrations compared to the control resulted in significantly lower body weights on D5.

The information provided resulted in an ACCEPTABLE classification using Mallard duck.

A dietary toxicity study using Mallard duck was conducted to determine the effect of Ethylenethiourea (ETU) on birds. A total of 10 animals per treatment were administered a diet of ETU for five days (D0-D5). The birds were observed for three days following exposure (Day 6-8). Measured concentrations (in parenthesis) as a percent of nominal were 95 (537 ppm a.i.), 97 (973 ppm a.i./L), 97 (1,780 ppm a.i./L), 97 (3,070 ppm a.i./L) and 98% (5,540 ppm a.i./L).

During acclimation, all birds were observed daily. Test birds were observed four times on the day of test initiation and twice daily through the rest of the test. Individual body weights were measured at test initiation (D 0), Day 5 and test termination (D 8).

No LC_{50} was calculated because no mortality was reported for any treatment group. Body weight data were compared using Bonferroni t-test in TOXSTAT® software.

Data Evaluation Report on the Acute Dietary Toxicity of Ethylenethiourea (Mancozeb-metabolite) with the Northern Bobwhite (*Colinus virginianus*)

EPA MRID Number 48417801

Data Requirement:

EPA DP Barcode	387914
EPA MRID	48417801
EPA Guideline	850.2200

Test material: Ethylenethiourea (Mancozeb metabolite) **Purity:** 100%
Common name Ethylenethiourea 2-Imidazolidinethione
Chemical name:
2-Imidazolidinenethione-Imidazoline-2-thiol
IUPAC: Ethylene thiourea
CAS: 2-imidazolidinethione
CAS No.: 96-45-7

Primary Reviewer: Christina deMariano **Date:** {05/05/2011}
EPA/OPP/EFED/EBR-5

Secondary Reviewer(s): {Valerie Woodard} **Date:** {11/17/2011}
{EPA/OECD/PMRA}

EPA PC Code 014504

Date Evaluation Completed: **05**-05-2011

CITATION: Hubbard, P.M. and Beaver, J.B. 2011. Ethylenethiourea: A dietary LC_{50} study with the northern bobwhite. Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Project No. 697-103. Study sponsored by EBDC/ETU Task force, Study number 2010-3. Study completion February 17, 2011 and submitted March 12, 2011. Day 0 of the in-life portion of the test initiated December 08, 2010. The in-life portion of the test was conducted from December 09, 2010 to December 17, 2010.

EXECUTIVE SUMMARY:

The acute dietary toxicity of Ethylenethiourea to 10-day old bobwhite quail (*Colinus virginianus*) was assessed over 8 days (5-day treatment period and 3-day post-treatment period). Ethylenethiourea was administered to the birds in the diet at nominal concentrations of 0 (negative control), 562, 1000, 1780, 3160 and 5620 mg ai/kg diet. Mean-measured concentrations were <12.5 (<LOQ, control), 537, 973, 1770, 3070 and 5540 mg ai/kg diet, respectively. Based on reductions in body weight gain at the 1780, 2980, and 5690 mg ai/kg, the NOEC was 1000 mg ai/kg. Because there were no effects at the highest tested concentration, the acute dietary LC_{50} could not be estimated. The acute dietary LC50 is expected to be >5620 mg ai/kg. According to the U.S. EPA classification, Ethylenethiourea (Ethylenethiourea 2-Imidazolidinethione) would be classified as practically non-toxic to northern bobwhite (*Colinus virginianus*) on an acute dietary basis.

There were no moralities in the control group and all control birds were normal in appearance and behavior throughout the test. In addition, there were no mortalities in the 562, 1000, 1780, 3160 and 5620 mg/kg.

There were no overt signs of toxicity at the 537, 973, or 1770 mg ai/kg test concentrations. One bird in the 537 kg ai/mg group was noted with a slightly swollen left foot from the afternoon of day 5 until test termination. Another bird was noted with lesions on the toes of the left foot during the day 8 body weight procedures, the probable result of cage mate aggression. Two birds in the 1770 mg ai/kg group were noted with toe lesions on day 5 of the test. One continued to be noted with lesions on the toes until the afternoon of day7 and the other until test termination. All other birds in the 537,

973, and 1770 mg ai/kg groups were normal in appearance and behavior throughout the test.

In the 3070 mg ai/kg group, one bird was noted with slight wing drop on day 5 of the test. The bird was normal in appearance and behavior for the remainder of the test. All other birds in the 3070 mg ai/kg group were normal in appearance and behavior throughout the test.

At the 5540 mg ai /kg test concentration, one bird was noted with a slight wing droop on the afternoon of day 4. A difference bird was noted with a slight wing droop on day 5 of the test. Also, another bird was noted with a slight ruffled appearance on day 5 and 6 of the test. All other birds were normal in appearance and behavior for the duration of the test and all birds were normal in appearance from the corning of day 7 until test termination.

No statistically difference in mean body weight or mean body weight change at the 537 and 973 mg ai/kg. For the 3070 and 5540 mg/kg test concentration, when compared to the control group, there were statistically significant ($p < 0.01$) reductions in body weight gain from day 0 to day 5 and in mean body weight on day 5. Also, from day 5 to day 8 there was a statistically significant reduction ($p < 0.05$) in mean body weight change at the 3070 mg ai/kg test concentration. When compared to control group, there were statistically significant ($p< 0.05$ or 0.01) reduction in the day 8 mean body weight at the 1770, 3070 and 5540 mg ai /kg concentrations. In addition, total body weight changes for the 3070 and 5540 mg/kg test concentration were reduced, with statistical significance of $p < 0.01$.

This toxicity study is classified as scientifically sound and is thus acceptable and does satisfy the guideline requirement for an acute dietary toxicity study for the Northern bobwhite quail.

Results Synopsis

Test Organism Age/Size (Mean Weight): 10-days old; 18 to 23 g (group means of 20.3 ± 2 g)

LC_{50}: Not estimated (> highest tested concentration of 5620 mg ai/kg diet)
Probit Slope: N/A 95% C.I.: N/A

NOEC: 973 mg ai/kg diet

Endpoint(s) affected: clinical signs of toxicity (i.e., subdued behavior, wing droop), body weight gains, and food consumption
Most sensitive endpoint(s): body weight gains

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA Pesticide Assessment Guidelines, OPPTS 850.2200

OECD Guideline 205, Avian Dietary Toxicity Test

Deviations from OPPTS 850.2200 included:

1. The relative humidity 15± 7%in the study was below the 850.2200 recommended ranges from 45 to 70%

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, August 1989; OECD Principles of GLP (ENV/MC/CHEM (98) 17.

A. MATERIALS:

1. Test Material	Ethylenethiourea 2-Imidazolidinethione
Description:	Solid
Lot No./Batch No. :	XW7-102353-014
Purity:	100%
Stability of compound under test conditions:	The stability of Ethylenethiourea in avian diet was assessed on day 5 in treated feed from all levels, with recoveries of 97 to 103% of nominal concentrations.

Physicochemical properties of ethylenethiourea

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species (common and scientific names): Bobwhite quail (*Colinus virginianus*)
(EPA recommends using either bobwhite quail or mallard duck.)

Age at study initiation: 10 days old
(EPA recommends: 10-14 days old for quail and 5-10 days old for mallards)

Weight at study initiation (mean and range): mean weight 20.3 ± 2 g and range of 18 to 23 g

Source: Wildlife International, Ltd. Easton, Maryland

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	10 days (pre-treatment period)	A detailed composition of the chick diet was provided.
Conditions: (same as test or not)	Same as test	*Test birds should be acclimated to test facilities and basal diet for at least 7 days.*
Feeding:	Throughout acclimation and testing all test birds were fed a game bird ration formulated to Wildlife International, Ltd. Specifications provided in appendix and domestic-quality potable water (town of Easton Maryland) were provided *ad libitum* during acclimation and duration of the test.	
Health: (any mortality observed)	Birds were in apparent good health and had received no medication. 12 of 206 birds died during 10- day acclimation period	
Pen size and construction materials	72 x 90 x 23 cm External walls, ceiling and floors were constructed of wire mesh and galvanized sheeting.	Birds were housed in groups of ten (by treatment). There was approximately 6480 cm^2 floor space per bird. *Recommended pen size is about 35 x 100 x 24 cm*
Test duration	10-day pre-treatment acclimation period; 5-day exposure period; and 3-day post-treatment period	*Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal:	0 (negative control), 562, 1000, 1780, 3160 or 5620 mg/bird/day	Measured concentrations were within ±2 .0% of nominal concentrations.
measured:	12.5 (<LOD, control), 537, 973, 1770, 3070, and 5540 mg//bird/day	*Five or six test concentrations should be used in a geometric scale, unless the LC_{50} > 5000 mg ai/kg diet.*
Solvent/vehicle, if used		

Parameter	Details	Remarks *Criteria*
type: amount:	N/A	*Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.*
Diet preparation and feeding	The portion of the ration and test material were placed in a Waring® blender. The weigh boat was rinsed with ration. Dry ice was placed on the outside of the blender and the contents were mixed for approximately one minute. The blender contents were added to the bowl and the blender was rinsed with ration, which was also added to the bowl and mixed for 15 minutes.	*The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.*
Feed withholding period	None	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	
Number of birds per replicate/groups for control: for treated:	 30 birds/replicate 10 birds/replicate	*The recommended number of birds per replicate is a minimum of ten.*
Number of replicates/group (if used) for control: for treated:	 6 replicates 2 replicate/level	
Test conditions temperature: relative humidity (%): photoperiod:	 **Brooding compartment** 39.0 ± 1.4°C (SD) **Average room temperature** 26 ± 1.0°C (SD) 15 ± 7% (mean) 16 light/8 dark	An infra-red heat source was suspended over each pen to provide additional heat. ***Recommended brooder temperature is*** *about 35°C (95°F)* ***Recommended room temperature is*** *22-27°C (71-81°F)* ***Recommended relative humidity is*** *45-70%* ***Recommended photoperiod*** *is a minimum of 14 hours of light.*
Reference chemical, if used	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	Mortality Clinical signs of toxicity Body weight Food consumption Necropsy	
Indicate the stability and homogeneity of test chemical in the diet	Stability: The stability of test material in avian diet was assessed on day 5 in treated feed from all levels, with recoveries of 97 to 103% of nominal concentrations. Homogeneity: homogeneity was assessed by collecting six samples each from the top left, top right, middle left, middle right, bottom right, bottom left middle from treated feed prepared at 562 and 5620 mg/kg diet. Coefficients of variation were 2.18 and 1.13%, respectively.	
Indicate if the test material was regurgitated	No regurgitation was indicated.	
Treatments on which necropsies were performed	No birds died in control group or due to treatment related effects.	Tissues examined included digestive tract, liver, kidneys, heart, spleen, muscle, and subcutaneous fat.
Observation intervals	Birds were observed for mortality and clinical signs of toxicity four times on Day 0, and twice daily throughout the remainder of the test. Group mean body weights were determined on Days 0, 5, and 8. Group mean food consumption was determined over Days 0 to 5 (daily), and 6 to 8.	
Were raw data included?	Yes, sufficient	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Table 3: Effect of Ethylenethiourea (Metabolite of Mancozeb) on Mortality of Bobwhite Quail.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.	No. of birds per treatment	Cumulative mortality						
		day 2	days 3	day 4	day 5	day 6	day 7	day 8
Negative control	30	0	0	0	0	0	0	0
562 (537)	10	0	0	0	0	0	0	0
1000 (973)	10	0	0	0	0	0	0	0
1780 (1770)	10	0	0	0	0	0	0	0
3160 (3070)	10	0	0	0	0	0	0	0
5620 (5540)	10	0	0	0	0	0	0	0
NOAEC				No mortality in tested concentration.				
LC_{50} (95% C.I.)				Not estimated due to any effects at highest tested concentration.				
Reference Chemical mortality				N/A				
Reference Chemical LC_{50}				N/A				
Reference Chemical NOAEC				N/A				

B. SUB-LETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed in birds from the control, 562, 1000 or 1780 ppm groups, or 5620 mg ai/kg diet levels. There was reduction in mean body weight gain from day 0 to 5 and in mean weight on day 5 for the 3160 and 5620 treatment groups. A reduction in mean body weight change was observed at 3160 mg ai/kg group when compared to the control on day 5 to day 8. Treatment groups 1780, 3160 and 5620 mg ai/kg when compared to the control had a reduction at test termination (day 8) in mean body weights. In addition, there was a statistically significant change in total body weight for the 3160 and 5620 mg ai/kg test concentration.

One bird at 562g ai/kg test concentration was noted with a slightly swollen left foot from the afternoon of Day 5 until test termination. In addition, another bird experienced lesions on the toes of the left foot during the day 8 weighting procedures, the probable result of cage mate aggression. Two birds at 1780 mg/kg group were noted with toe lesions on day 5. One continued to be noted with toe lesions until the afternoon day 7 and the other until test termination.

In the 3160 mg/kg group, one bird was noted with slight wing droop on day 5. The bird was normal in appearance and behavior for the remainder of the test.

In the 5620 mg/kg group, one bird was noted with a slight wing droop on the afternoon of day 4. A difference bird, in the same group, was noted with a slight wing droop on day 5. Also, another bird was noted with a slight riffled appearance on day 5 and 6 of the test.

Table 4: Sublethal Effect of Ethylenethiourea (metabolite of Mancozeb) on Bobwhite Quail.

Treatment, mg/kg diet Mean-measured (and nominal) conc.		Observation				
		Mean Body weight, g			Food consumption, g/bird/day	
	Day 0	Day 5	Day 8	Total change	Days 0 to 5 (Exposure Period)	Days 6-8 (Post-Exposure Period)
Negative control	21.0	31.0	42	21.0	6.0	8.0
562 (537)	20.0	30.0	38	17.0	6.0	7.0
1000 (973)	21.0	31.0	40	20.0	6.0	7.0
1780 (1770)	20.0	28.0	37*	17.0*	6.0	7.0
3160 (3070)	20.0	25.00**	34**	13.00**	5.0	7.0
5620 (5540)	20.0	23.00**	31.00**	12.00**	7.0	6.0
NOEC		1000 mg ai/kg diet			Not reported	
LC_{50}		Not reported			Not reported	
Reference chemical N		N/A			N/A	
Reference chemical EC_{50}		N/A			N/A	
	* Statistically different from the control group p<0.05 ** Statistically different from the control group p<0.01					

C. REPORTED STATISTICS:

There were no treatment related mortalities observed in this study. Therefore, no statistical analyses were performed for mortality data.

LC_{50}: NA 95% C.I.: N/A
NOAEC: 1000 mg ai/kg

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Raw data was provided for body weight to statistically verify the study author's findings. The reviewer concluded that there is a significant reduction in body weight in the 562 mg/kg treatment group. At the next higher concentration of, 1000 mg/kg body weight was not significantly reduced. This is a biological indication of a non-dose related, non-treatment effect. Due to this the reviewer agrees with study author of a NOEC of 1000 mg ai/kg. At the highest treatment group, 5620 mg/kg, body weight was reduced by 26% by day 8.

NOAEC: 1000 mg ai/kg diet

Endpoint(s) affected: Body weight change (most sensitive) and sublethal effects (i.e., subdued behavior and wing droop)

E. STUDY DEFICIENCIES:

1. The relative humidity 15± 7% in the study was below the 850.2200 recommended range of 45 to 70%

F. REVIEWER'S COMMENTS:

The reviewer's conclusion regarding the NOAEC for body weight change was the same as study authors. It must be noted that there was significant body weight reduction in the 562 mg/kg group but at next highest levels, 1000 mg/kg, there was no significant body weight reduction. There was also significant weight reduction at the highest concentrations of 1780, 3160 and 5620 mg/kg.

Experimental test dates were December 8 to 17, 2011

G. CONCLUSIONS:

This study is classified as Acceptable and satisfies the guideline. Based on reductions in body weight change at the 1780, 3160 and 5620 mg ai/kg diet levels, the NOAEC is 1000 mg ai/kg diet. No adverse signs of toxicity at the 562 or 1000 mg ai/kg test concentrations were observed. Sublethal effects where observed such as wing droop in birds in the 3,160 mg/kg and 5,620 mg/kg treatment groups, respectively. Birds appeared normal at test termination, day 8. Birds, in 562 and 1,780 mg/kg test group, were noted with toe lesions. It was suggested that this could be due to cage mating aggression. No treatment-related mortality or post-mortem abnormalities were observed. The acute dietary LC_{50} was not estimated because no mortality was observed at the highest tested concentration.

LC_{50}: Not estimate	95% C.I.: N/A
Probit Slope: N/A	95% C.I.: N/A

NOAEC: 1000 mg ai/kg diet

Endpoint(s) affected: clinical signs of toxicity (i.e., subdued behavior), body weight gains, and food consumption
Most sensitive endpoint(s): body weight gains

Statistically output of change in weigh of mallards

Title: Northern Bobtail weight change, Ethylenethiourea, day 5
File: bob5
Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	743.4833	148.6967	13.6171
Within (Error)	74	808.0667	10.9198	
Total	79	1551.5500		

(p-value = 0.0000)

Critical F = 3.2752 (alpha = 0.01, df = 5,74)
= 2.3383 (alpha = 0.05, df = 5,74)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Northern Bobtail weight change, Ethylenethiourea, day 5
File: bob5
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	control	31.4667	31.4667		
2	562 mg/kg	29.5000	29.5000	1.6299	
3	1000 mg/kg	29.9000	29.9000	1.2984	
4	1780 mg/kg	27.8000	27.8000	3.0387	*
5	3160 mg/kg	25.0000	25.0000	5.3592	*
6	5620 mg/kg	22.8000	22.8000	7.1825	*

Bonferroni t critical value = 2.3778 (1 Tailed, alpha = 0.05, df = 5,74)

Title: Northern Bobtail weight change, Ethylenethiourea, day 5
File: bob5
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	30			
2	562 mg/kg	10	2.8691	9.1	1.9667
3	1000 mg/kg	10	2.8691	9.1	1.5667
4	1780 mg/kg	10	2.8691	9.1	3.6667
5	3160 mg/kg	10	2.8691	9.1	6.4667
6	5620 mg/kg	10	2.8691	9.1	8.6667

Title: Northen bobtail weight change, ethylenethiourea - day 8
File: bob8
Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	1121.4875	224.2975	14.5151
Within (Error)	74	1143.5000	15.4527	
Total	79	2264.9875		

(p-value = 0.0000)

Critical F = 3.2752 (alpha = 0.01, df = 5,74)
= 2.3383 (alpha = 0.05, df = 5,74)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Northen bobtail weight change, ethylenethiourea - day 8
File: bob8
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED ORIGINAL UNITS	IN t STAT	SIG 0.05
1	control	42.2000	42.2000		
2	562 mg/kg	37.5000	37.5000	3.2744	*
3	1000 mg/kg	40.3000	40.3000	1.3237	
4	1780 mg/kg	37.0000	37.0000	3.6227	*
5	3160 mg/kg	33.5000	33.5000	6.0610	*

6	5620 mg/kg	32.2000	32.2000	6.9667	*

Bonferroni t critical value = 2.3778 (1 Tailed, alpha = 0.05, df = 5,74)

Title: Northen bobtail weight change, ethylenethiourea - day 8
File: bob8
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	30			
2	562 mg/kg	10	3.4131	8.1	4.7000
3	1000 mg/kg	10	3.4131	8.1	1.9000
4	1780 mg/kg	10	3.4131	8.1	5.2000
5	3160 mg/kg	10	3.4131	8.1	8.7000
6	5620 mg/kg	10	3.4131	8.1	10.0000

III. REFERENCES:

A reference list was not provided.

Data Evaluation Report on the Acute Dietary Toxicity of Ethylenethiourea (Mancozeb-metabolite) with the Mallard (*Anas platyrhynchos*)

EPA MRID Number 48417802

Data Requirement:

EPA DP Barcode	387914
EPA MRID	48417802
EPA Guideline	850.2200

Test material: Ethylenethiourea (Mancozeb metabolite) **Purity:** 100%
Common name Ethylenethiourea 2-Imidazolidinethione
Chemical name:
2-Imidazolidinenethione-Imidazoline-2-thiol
IUPAC: Ethylene thiourea
CAS: 2-imidazolidinethione
CAS No.: 96-45-7

Primary Reviewer: Christina deMariano **Date:** {.....}
EPA/OPP/EFED/EBR-5

Secondary Reviewer(s): {.....} **Date:** {11/18/2011}
{EPA/OECD/PMRA}

EPA PC Code 014504

Date Evaluation Completed: 10-31-2011

CITATION: Hubbard, P.M. and Beaver, J.B. 2011.Ethlenethiourea: A dietary LC_{50} study with the Mallard. Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Project No. 697-104. Study sponsored by EBDC/ETU Task force study number 2010-4. Study initiated December 08, 2010, completion December 17, 2010 and submitted March 12, 2011.

EXECUTIVE SUMMARY:

The acute dietary toxicity of Ethylenethiourea to 10-day old mallard (*Anas platyrhynchos*) was assessed over 8 days (5-day treatment period and 3-day post-treatment period). Ethylenethiourea was administered to the birds in the diet at nominal concentrations of 0 (negative control), 562, 1000, 1780, 3160, and 5620 mg ai/kg diet. Mean-measured concentrations were <12.5 (<LOD, control), 537, 973, 1770, 3070 and 5540 mg ai/kg diet, respectively. Based on reductions in body weight gain at the 1760, 2980, and 5690 mg ai/kg, the NOEC was < 562 mg ai/kg. The acute dietary LC_{50} >5540 (5620 nominal) mg ai/kg. According to the U.S. EPA classification, Ethylenethiourea (Ethylenethiourea 2-Imidazolidinethione) would be classified as practically non-toxic mallard (*Anas platyrhynchos*) on an acute dietary basis.

There were no treatment-related moralities. All birds in the control group and all birds in all treatment groups were normal in appearance and behavior for the duration of the test.

When compared to the control group, there was statistically significant ($p<0.01$) reduction in mean body weight at all the test concentrations from day 0 to day 5. Day 5 mean body weights were statistically ($p< 0.05$ or 0.01) less for all the test concentrations when compared to the control group. The mean body weight change for the 973, 1770, and 5540 mg ai/kg test concentrations from day 5 to day 8 was statistically ($p < 0.05$ or 0.01) less than the control mean body weight change. Day 8 mean body weights were statistically ($p<0.01$) less for the 973, 1770, 3070 and 5540 mg ai/kg test concentrations when compared to the control group. The mean total body weight change was statistically ($p < 0.05$ or 0.01) less for all of the test concentrations when compared to the control group.

When compared to the control group, there were no apparent treatment related effects on feed consumption at the 537, 973, 1770, 3070 and 5540 mg ai/kg treatment groups.

This toxicity study is classified as scientifically sound and is thus acceptable to satisfy the guideline requirement for an acute dietary toxicity study for the mallard (*Anas platyrnchos*).

Results Synopsis

Test Organism Age/Size (Mean Weight): 10-days old; 138 to181 g

LC_{50}: >5620 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOEC: Effects reported at lowest treatment concentration. (< 562 mg ai/kg diet)

Endpoint(s) affected: clinical signs of toxicity (i.e., subdued behavior, wing droop), body weight gains, and food consumption
Most sensitive endpoint(s): body weight gains.

MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA Pesticide Assessment Guidelines, OPPTS 850.2200

OECD Guideline 205, Avian Dietary Toxicity Test

Deviations from OPPTS 850.2200 included:

1. The relative humidity30 ± 8% in the study was below the 850.2200 recommended ranges from 45 to 70%

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, August 1989; OECD Principles of GLP (ENV/MC/CHEM (98) 17.

A. MATERIALS:

1. Test Material Ethylenethiourea 2-Imidazolidinethione

Description: Solid

Lot No./Batch No. : XW7-102353-014

Purity: 100%

Stability of compound under test conditions: The stability of Ethylenethiourea in avian diet was assessed on day 5 in treated feed from all levels, with recoveries of 86 to 95% of nominal

concentrations.

Physicochemical properties of ethylenethiourea

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species (common and scientific names): Mallard Duck (*Anas Platyrhynchos)*
(EPA recommends using either bobwhite quail or mallard duck.)

Age at study initiation: 10 days old
(EPA recommends: 10-14 days old for quail and 5-10 days old for mallards)

Weight at study initiation (mean and range): mean weight 165 g and range of 138 to 181 g

Source: Wildlife International, Ltd. Easton, Maryland

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period:	8 days (pre-treatment period)	A detailed composition of the chick diet was provided.
Conditions: (same as test or not)	Same as test	*Test birds should be acclimated to test facilities and basal diet for at least 7 days.*
Feeding:	Throughout acclimation and testing all test birds were fed a game bird ration formulated to Wildlife International, Ltd. specifications provided in appendix. Domestic-quality potable water (town of Easton Maryland) was provided *ad libitum* during acclimation and duration of the test.	
Health: (any mortality observed)	Birds were in apparent good health and had received no medication. No moralities during the 8- day acclimation period	
Pen size and construction materials	62 x 92 x 25.5 cm External walls, ceiling and floors were constructed of vinyl coated wire grid.	Birds were housed in groups of ten (by treatment). There was approximately 5704cm^2 floor space per bird. *Recommended pen size is about 35 x 100 x 24 cm*
Test duration	8-day pre-treatment acclimation period; 5-day exposure period; and 3-day post-treatment period	*Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal:	0 (negative control), 562, 1000, 1780, 3160 and 5620 mg/bird/day	Measured concentrations were within ±2 .0% of nominal concentrations.
measured:	12.5 (<LOD, control), 537, 973, 1770, 3070, and 5540 mg//bird/day	*Five or six test concentrations should be used in a geometric scale, unless the LC_{50} > 5000 mg ai/kg diet.*
Solvent/vehicle, if used		

Parameter	Details	Remarks *Criteria*
type: amount:	N/A	*Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.*
Diet preparation and feeding	The portion of the ration and test material were placed in a Waring® blender. The weigh boat was rinsed with ration. Dry ice was placed on the outside of the blender and the contents were mixed for approximately one minute. The blender contents were added to the bowl and the blender was rinsed with ration, which was also added to the bowl and mixed for 15 minutes.	*The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.*
Feed withholding period	None	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	
Number of birds per replicate/groups for control: for treated:	 30 birds/replicate 10 birds/replicate	*The recommended number of birds per replicate is a minimum of ten.*
Number of replicates/group (if used) for control: for treated:	 6 replicates 2 replicate/level	
Test conditions temperature: relative humidity (%): photoperiod:	 **Brooding compartment** 30.1 ± 1.3°C (SD) **Average room temperature** 23.7 ± 0.4°C (SD) 30 ± 8% (mean) 16 light/8 dark	An infra-red heat source was suspended over each pen to provide additional heat. ***Recommended brooder temperature is*** *about 35°C (95°F)* ***Recommended room temperature is*** *22-27°C (71-81°F)* ***Recommended relative humidity is*** *45-70%* ***Recommended photoperiod*** *is a minimum of 14 hours of light.*
Reference chemical, if used	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	Mortality Clinical signs of toxicity: Body weight Food consumption Necropsy	
Indicate the stability and homogeneity of test chemical in the diet	Stability: stability of the test material in avian diet was assessed in all test concentrations at day 5, with recoveries of 86 to 95%. Homogeneity: homogeneity was assessed by collecting six samples each from the top left, top right, middle left, middle right, bottom right, bottom left middle from treated feed prepared at 537 and 5540 mg/kg diet, with recovery of 96 and 99%, respectively. Coefficients of variation were 2.18 and 1.13%, respectively.	
Indicate if the test material was regurgitated	No regurgitation was indicated.	
Treatments on which necropsies were performed	No birds died in control group or due to treatment related effects.	Tissues examined included digestive tract, liver, kidneys, heart, spleen, muscle, and subcutaneous fat.
Observation intervals	Birds were observed for mortality and clinical signs of toxicity four times on Day 0, and twice daily throughout the remainder of the test. Group mean body weights were determined on Days 0, 5, and 8. Group mean food consumption was determined over Days 0 to 5 (daily), and 6 to 8.	
Were raw data included?	Yes, sufficient	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Table 3: Effect of Ethylenethiourea (Metabolite of Mancozeb) on Mortality of Mallard Duck.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.	No. of birds per treatment	Cumulative mortality						
		day 2	days 3	day 4	day 5	day 6	day 7	day 8
Negative control	30	0	0	0	0	0	0	0
562 (537)	10	0	0	0	0	0	0	0
1000 (973)	10	0	0	0	0	0	0	0
1780 (1770)	10	0	0	0	0	0	0	0
3160 (3070)	10	0	0	0	0	0	0	0
5620 (5540)	10	0	0	0	0	0	0	0
NOAEC				>562 mg ai/kg diet				
LC_{50} (95% C.I.)				No mortalities in any group. <5620 mg ai/kg diet				
Reference Chemical: mortality				N/A				
Reference Chemical: LC_{50}				N/A				
Reference Chemical: NOAEC				N/A				

B. SUB-LETHAL TOXICITY ENDPOINTS:

There was no statistically significant reduction in mean body weight gain for any test concentrations from day 0 to day 5 when compared to control group. Day 5 mean body weights were significantly ($p<0.05$ or 0.01) less for all the test concentrations when compared to the control group. The mean body change for the 973, 1770, and 5540 mg ai/kg test concentrations from day 5 to day 8 was statistically ($p<0.05$ or 0.01) less than the control mean body weight change. Day 8 mean body weight were significantly ($p< 0.01$) less for the 973, 1770, 3070 and 5540 mg/kg test concentration when compared to the control groups. The mean total body weight change was statistically less ($p< 0.05$ or 0.01) for all of the test concentrations when compared to the control group.

When compared to the control group, there were no apparent related effects on feed consumption at the 537, 973, 1770, 3070 and 5540 mg/kg treatment groups.

Table 4: Sublethal Effect of Ethylenethiourea (metabolite of Mancozeb) on Mallard Duck.

Treatment, mg/kg diet Mean-measured (and nominal) conc.		Observation				
		Mean Body weight, g			Food consumption, g/bird/day	
		Days 0	Days 5	Total change	Days 0 to 5 (Exposure Period)	Days 6-8 (Post-Exposure Period)
Negative control		162.0	303	246	103.0	149.0
563 (537)		167.0	278*	218*	109.0	165.0
1000 (973)		171.0	273**	180**	93.0	120.0
1780 (1770)		169.0	274**	190**	98.0	128.0
3160 (3070)		165.0	277*	204**	117.0	204.0
5620 (5540)		166.0	263**	185**	96.0	143.0
NOEC		>537 mg ai/kg diet			Not reported	
LC_{50}		Not reported			Not reported	
Reference chemical	NOAEC	N/A			N/A	
	EC_{50}	N/A			N/A	

* Visually determined to be a treatment-related difference compared to the controls.

C. REPORTED STATISTICS:

There were no treatment related mortalities observed in this study. Therefore, no statistical analyses were performed for mortality data.

LC_{50}: >5620 mg ai/kg 95% C.I.: N/A
NOAEC: < 562 mg ai/kg

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: TOXSTAT Bonferroni t-test. The reviewer conclusions were the same as study author. There may have been a treatment-related reduction (15%) in body weight change of birds at the end of the 8 days at the 5540 mg ai/kg diet level. As a result, the reviewer visually determined the NOAEC to be less than 537 mg/kg. The study author's NOAEC for food consumption could be visually verified.

LC_{50}: Not calculated (>5620 mg ai/kg diet) 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC: >562 mg ai/kg diet

Endpoint(s) affected: Body weight change (most sensitive) and sublethal effects (i.e., subdued behavior and wing droop)
Most sensitive endpoint: Weight loss

E. STUDY DEFICIENCIES:

1. The relative humidity 30 ± 8% in the study was below the 850.2200 recommended range of 45 to 70%. This did not affect the acceptability of the study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusion regarding the NOAEC for body weight change was the same as the study author. Experimental test dates were December 8 to 17, 2010.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. Based on reductions in body weight change at the 973, 1770, 307 and 5540 mg ai/kg diet levels, the NOAEC is 537 mg ai/kg diet. No treatment-related mortality or post-mortem abnormalities were observed. The acute dietary LC_{50} was not calculated due to no mortality in any concentration groups (>5620 mg ai/kg diet).

LC_{50}: >5620 mg ai/kg diet 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

NOAEC: >562 mg ai/kg diet

Endpoint(s) affected: clinical signs of toxicity (i.e., subdued behavior), body weight gains, and food consumption
Most sensitive endpoint(s): body weight gains

Title: weight change in mallard, ethylenethiourea-day5
File: mallard5
Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	22142.7208	4428.5442	6.3761
Within (Error)	74	51397.1667	694.5563	
Total	79	73539.8875		

(p-value = 0.0001)

Critical F = 3.2752 (alpha = 0.01, df = 5,74)
= 2.3383 (alpha = 0.05, df = 5,74)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: weight change in mallard, Ethylenethiourea – day 5
File: mallard5
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	control	306.1333	306.1333		
2	562 mg/kg	277.8000	277.8000	2.9442	*
3	1000 mg/kg	273.4000	273.4000	3.4015	*
4	1780 mg/kg	273.6000	273.6000	3.3807	*
5	3160 mg/kg	276.6000	276.6000	3.0689	*
6	5620 mg/kg	262.9000	262.9000	4.4926	*

Bonferroni t critical value = 2.3778 (1 Tailed, alpha = 0.05, df = 5,74)

Title: weight change in mallard, Ethylenethiourea - day 5
File: mallard5
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	30			
2	562 mg/kg	10	22.8823	7.5	28.3333
3	1000 mg/kg	10	22.8823	7.5	32.7333
4	1780 mg/kg	10	22.8823	7.5	32.5333
5	3160 mg/kg	10	22.8823	7.5	29.5333
6	5620 mg/kg	10	22.8823	7.5	43.2333

Title: weight change in mallard, Ethylenethiourea - day 8
File: mallard8
Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	47287.6875	9457.5375	9.9377
Within (Error)	74	70424.5000	951.6824	
Total	79	117712.1875		

(p-value = 0.0000)

Critical F = 3.2752 (alpha = 0.01, df = 5,74)
= 2.3383 (alpha = 0.05, df = 5,74)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: weight change in mallard, Ethylenethiourea - day 8
File: mallard8
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05	
1	control	408.3000	408.3000			
2	562 mg/kg	384.9000	384.9000	2.0773		
3	1000 mg/kg	350.9000	350.9000	5.0956	*	
4	1780 mg/kg	358.8000	358.8000	4.3943	*	
5	3160 mg/kg	368.6000	368.6000	3.5243	*	
6	5620 mg/kg	350.4000	350.4000	5.1400	*	

Bonferroni t critical value = 2.3778 (1 Tailed, alpha = 0.05, df = 5,74)

Title: weight change in mallard, Ethylenethiourea - day 8
File:
mallard8
Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	control	30			
2	562 mg/kg	10	26.7850	6.6	23.4000
3	1000 mg/kg	10	26.7850	6.6	57.4000
4	1780 mg/kg	10	26.7850	6.6	49.5000
5	3160 mg/kg	10	26.7850	6.6	39.7000
6	5620 mg/kg	10	26.7850	6.6	57.9000

I. REFERENCES:

A reference list was not provided.